

# The Effects of Prolonged Strenuous Exercise on Salivary Secretion of IgA Subclasses in Men

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## Abstract

Low levels of secretory IgA (sIgA) have been associated with recurrent upper respiratory tract infection (URTI). Saliva contains approximately 60% sIgA1 and 40% sIgA2 in normal adults. Because sIgA1 is more susceptible to proteases degradation produced by mucosal pathogens, the higher the proportion of sIgA1, may increase the risk of bacterial mucosal infection. The aim of the present study is to investigate the effects of acute prolonged intensive exercise on saliva output rate and salivary concentrations of sIgA1 and sIgA2. Following approval by the local Ethics Committee, eight male volunteers (age  $21.3 \pm 1.0$  years, body mass  $66.8 \pm 2.0$  kg,  $\dot{V}O_{2max}$   $52.5 \pm 2.1$  mL·kg<sup>-1</sup>·min<sup>-1</sup>, peak power  $259 \pm 6$  watt; means  $\pm$  SEM) performed either a single bout of cycling at 55% peak power for 2 h (started at 9:00) or a separate resting trial separated by at least a week in a counterbalanced design. Timed, unstimulated saliva samples and venous blood samples were obtained at 10 min before exercise (PreEX), after 58-60 min (Mid-EX, saliva only) and during the last 2 min of exercise (Post-EX), and at 1 h (P-EX-1h), and 2 h (P-EX-2h, saliva only) after exercise. The concentrations of sIgA and sIgA1 were determined by ELISA. The results showed that a 2-h cycling at 55% peak power significantly decreased blood glucose concentration ( $P < 0.01$ ) and increased plasma concentrations of cortisol and adrenaline (both  $P < 0.01$ ). However, there were no significant effects on saliva flow rate, sIgA level, and sIgA1/sIgA ratio. These findings suggested that a single bout of cycling at 55% peak power for 2 h does not appear to acutely affect oral immunity.

**Keywords:** Exercise, SIgA subclass, Stress hormones

## Introduction

Many studies have shown that various aspects of immune function are temporarily changed following exercise [1]. Prolonged strenuous exercise results in a temporary immune suppression that typically lasts for 3 to 24 h after exercise [2]. Athletes appear to have a higher incidence of upper respiratory tract infection (URTI) than sedentary counterparts during and after scheduled training sessions or competition periods [3, 4].

Secretory immunoglobulin A (sIgA) is the predominant immunoglobulin found in the secretions lining mucosal surfaces and is an important component of saliva [5]. Secretory IgA is produced in local plasma cells and seems to function as a multi-layered mucosal defense. For example, IgA prevents antigens and microbes from adhering to and penetrating the epithelium (immune exclusion), interrupts replication of intracellular pathogens during transcytosis through epithelial cells (intracellular neutralisation), and binds antigens in the lamina propria facilitating their excretion through the epithelium back into the lumen (immune excretion) [6]. Mucosal IgA is also a major mediator of nasal immunity [7]. Lower levels of salivary IgA (sIgA) or chronic sIgA deficiency have been associated with an increased frequency of URTI

episodes [8], recurrent URTI, or reduced protection against certain epithelial infections [9]. It may be due to the low sIgA level allowing pathogens to enter body tissues via the epithelial surface [10].

The relationship between susceptibility to URTI and exercise workload is modeled as a J-shaped curve [11]. It predicts that individuals who exercise moderately have a lower risk of infection, whereas those who exercise heavily have a higher risk compared with sedentary counterparts. Preliminary evidence has been provided by many previous studies [8, 12, 13]. Numerous studies have examined how the sIgA concentration or secretion rate is affected by exercise. However, the results have been inconsistent to date. Some studies showed sIgA concentration was depressed after strenuous exercise [14-16], whereas other studies reported sIgA to be unaffected [17-19] or even elevated [20-22]. Some recent studies reported that salivary flow rate and sIgA secretion rate, but not sIgA concentration, were reduced following a triathlon race [23] or tennis drills [24]. However, several studies have also shown a stable secretion rate of sIgA following tennis drills [25], soccer match [26], or cycling [20]. Jemmott and McClelland [27] concluded from a meta-analysis of nine studies that the level of IgA secretion may indicate the vulnerability toward URTI. Mackinnon and Hooper [28] further suggested that the protective effect might not only depend on sIgA concentration but also on salivary flow rate.

In humans, IgA occurs as two subclasses that differ in amino acid sequence and glycosylation of the alpha heavy chain [29]. Subclass IgA1 predominates (~90%) in serum, whereas IgA2

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predominates in most mucosal secretions [30]. The proportions of the two subclasses vary between mucosal sites, due to difference in the distribution of immunoglobulin-producing immunocytes [31]. Saliva contains approximately 60% IgA1 in the normal adults [32].

IgA1 is primarily produced in response to protein antigens, while IgA2 is induced by carbohydrates and lipid-containing antigens [31]. While the majority of the respiratory infections were of viral aetiology, bacterial infections were also detected and the role IgA1 proteases may be important in these cases. The IgA1 proteases produced by most bacterial mucosal pathogens degrade IgA1 antibodies, while IgA2 antibodies are protected, a fact that is important when considering resistance to infections at mucosal surfaces. Because IgA1 is more susceptible to the proteases produced by mucosal pathogens, the higher proportion of IgA1 and the lower IgA2 may increase the risk for bacterial mucosal infection. IgA2 is more resistant than IgA1 to most bacterial IgA proteases [33] and therefore may play a more important role in protecting mucosal surfaces against microbial infections.

Two studies have been reported the influences of exercise on IgA subclasses in human [8, 34]. One of them showed that total IgA and IgA1, but not IgA2, concentrations in breast milk were decreased after exhaustive exercise, with recovery to baseline levels within 60 min [34]. Another one was reported that the salivary IgA1 concentration in the early phase of the training season were significantly associated with the number of URTI during the training season in elite swimmers and the proportion of IgA1 in the saliva of the elite swimmers was higher than for normal sedentary counterparts (80% vs. 60%) [35]. Since, up-to-date, no study has been done to investigate the relationship between an acute prolonged intense exercise and the level of sIgA subclasses in man. Therefore, the purposes of the present study was to determine the effect of an acute bout of 2-hr cycling at 55% peak power on saliva flow rate, salivary concentrations of IgA and IgA1 and plasma concentrations of adrenaline and cortisol.

## Methods

### Subjects:

Eight male volunteers (age  $21.3 \pm 1.0$  years, body mass  $66.8 \pm 2.0$  kg,  $\dot{V}O_{2\max}$   $52.5 \pm 2.1$  mL·kg<sup>-1</sup>·min<sup>-1</sup>, peak power  $259 \pm 6$  watt; means  $\pm$  SEM), who were recreationally active and familiar with cycling, participated in the study. After receiving written information and passing a Health Questionnaire screen, subjects gave written informed consent. Subjects were requested to complete a dietary record sheet on the day prior to Trial 1 and then repeat the same diet according to the dietary record before Trial 2. Subjects were also asked not to perform any strenuous exercise or consume alcohol or medication for 2 days before each trial. The protocol was approved by the Ethics Committee of our university before the study began.

### Preliminary Measurements

Maximal oxygen uptake was estimated by means of a continuous incremental exercise test on a cycle ergometer (Monark 874E, Monark Exercise AB, Sweden) to volitional exhaustion. Participants began cycling at 70 W with increments of 35 W every 3 min. The cadence remained at 70 rev·min<sup>-1</sup> and heart rate was monitored using radiotelemetry. During the third minute of each work rate increment, expired gas was collected in Douglas bags. An oxygen/carbon dioxide analyzer (Servomex 1400B, Crowborough, UK) was used along with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for determination of  $\dot{V}O_2$  and  $\dot{V}CO_2$ .

### Experimental Procedure

Subjects performed 2 h cycling (started at 09:00) at 55%

peak aerobic power ( $143 \pm 4$  W) or a separate resting control trial in a counterbalanced order after an overnight fast, separated by at least 6 days. Timed, unstimulated saliva samples and venous blood samples were obtained at 10 min before exercise (preEX), after 58-60 min (midEX, saliva only) and during the last 2 min of exercise (postEX), and at 1 h (1h-postEX), and 2 h (2h-postEX, saliva only) after exercise in cycling trial and at the corresponding timepoints in resting control trial. Participants were asked to empty the bladder before measurement of body mass, and performed cycling at 70 rev·min<sup>-1</sup> on the same ergometer used to determine  $\dot{V}O_{2\max}$ . Heart rate was recorded continuously during exercise by radiotelemetry. Ratings of perceived exertion (RPE) were obtained at 15-min intervals. No food was consumed before trials finished and water ingestion was allowed *ad libitum* during the trials except for the 5 min preceding each saliva sample collection. The laboratory temperature and relative humidity were  $20.2 \pm 1.0^\circ\text{C}$  and  $65 \pm 5\%$ , respectively.

### Saliva Collection and Analysis

Participants were seated during all saliva collections. With an initial swallow to empty the mouth, unstimulated whole saliva was collected by expectoration into a pre-weighed vial (7ml-capacity plastic Bijou tubes with screw top) for 2 min with eyes open, head tilted slightly forward and making minimal orofacial movement. All saliva samples were stored at  $-20^\circ\text{C}$  until analysis.

Saliva flow rate (mL·min<sup>-1</sup>) was determined by weighing. The density of saliva was assumed to be 1.0 g·mL<sup>-1</sup> [36]. The concentration of salivary IgA, IgA1 (mg·L<sup>-1</sup>) were determined by a sandwich-ELISA method similar to that described by [37]. Briefly, flat-bottomed microtitration plates (Costar EIA/RIA plate, Sigma, Poole, UK) were coated with the primary antibody, rabbit anti-human IgA (I-8760, Sigma) or IgA1 (I-7262, Sigma), at a dilution of 1 in 800 in carbonate buffer, pH 9.6, and kept at 4°C over night. After washing with phosphate buffered saline (PBS, pH 7.2) the plates were coated with blocking protein solution (2 g·L<sup>-1</sup> bovine serum albumin in PBS). Sample analysis was performed in triplicate using saliva samples diluted 1 in 500 with deionised water. A range of standards (Human colostrum IgA (I-2636) and IgA1 (I-4123), Sigma) up to 600 µg·L<sup>-1</sup> is used for calibration. Standards were incorporated into each micro-well plate, and all samples from a single subject were analyzed on a single plate. The plates were incubated for 90 min at room temperature. Following a washing step, peroxidase-conjugated goat anti-human IgA (A-4165, Sigma) is added and the plate incubated for a further 90 min at room temperature. Following another washing step, the substrate, ABTS (Boehringer Mannheim, Lewes, UK), is added and after 30 min the absorbance was measured at 405 nm.

The secretion rate (µg·min<sup>-1</sup>) of sIgA and sIgA1 was calculated by multiplying the concentrations of sIgA and sIgA1, respectively, by the saliva flow rate.

### Blood Collection and Analysis

Venous blood samples were taken from an antecubital vein by venepuncture, and were collected into Vacutainer tubes (Becton Dickinson, Oxford, UK) containing K<sub>3</sub>EDTA. Blood was subsequently spun at 3000 g for 10 min at 4 °C within 10 min of sampling. The plasma obtained was immediately stored at  $-20^\circ\text{C}$  prior to analysis. Plasma aliquots were analyzed to determine the concentrations of adrenaline (IBL GmbH, Hamburg) and cortisol (DRG Instruments GmbH, Germany) using enzyme-linked immunosorbent assay (ELISA) kits whereas glucose concentrations were determined using GOD-PAP method (Randox, Ireland). The intra-assay coefficient of variation was 1.3%, 12.7%, and 6.9% for glucose, adrenaline, and cortisol, respectively.

### Statistical Analysis

All results were presented as mean values and standard errors of the mean ( $\pm$ SEM). Data were checked for normality, homogeneity of variation and sphericity before statistical analysis, and the Huynh-Feldt method was applied for adjustment of degree of freedom for the  $F$ -tests. Data were analysed using a two-factor (trial  $\times$  time) repeated measures ANOVA with *post hoc* Tukey tests and paired  $t$  tests applied where appropriate.  $P < 0.05$  was accepted significant.

### Results

#### Glucose and Stress Hormones

There was significant main effect of time ( $F_{3,21} = 4.51$ ;  $P = 0.026$ ) for plasma glucose concentration, which was lower at P-EX-1h than Pre-EX in EX trial (Figure 1).

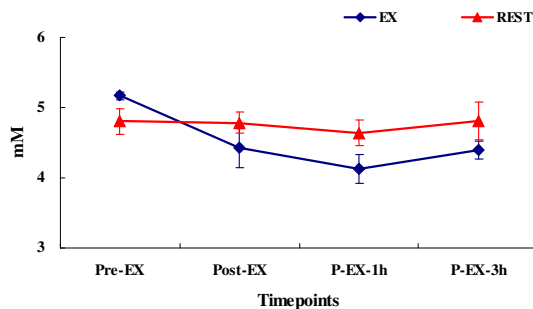


Figure 1. Changes in concentrations of plasma glucose. Values are means  $\pm$  SEM ( $n = 8$ ). Significantly different from Pre-EX in EX trial ( $^{aa}P < 0.01$ ).

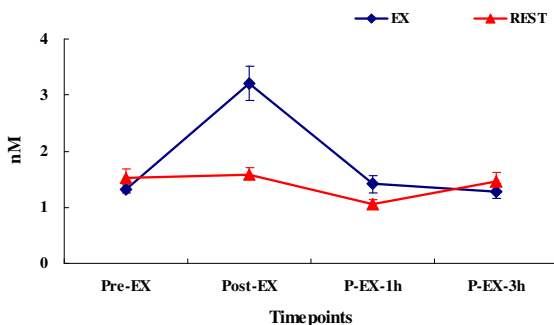


Figure 2. Changes in concentrations of plasma adrenaline. Values are means  $\pm$  SEM ( $n = 8$ ). Significantly different from Pre-EX in EX trial ( $^{aa}P < 0.01$ ); significantly different between trials ( $^{**}P < 0.01$ ).

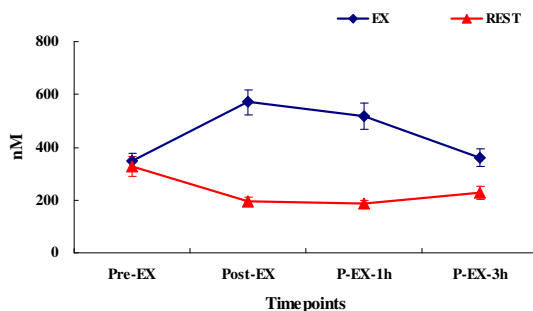


Figure 3. Changes in concentrations of plasma cortisol. Values are means  $\pm$  SEM ( $n = 8$ ). Significantly different between trials ( $^{**}P < 0.01$ ).

There were significant main effects of trial ( $F_{1,7} = 6.27$ ;  $P = 0.041$ ), time ( $F_{3,21} = 36.23$ ;  $P < 0.001$ ), and interaction between trial and time ( $F_{3,21} = 16.26$ ;  $P < 0.001$ ) for plasma concentrations of adrenaline, with higher levels at Post-EX compared with Pre-EX in EX trial and the same timepoint in REST trial (Figure 2).

There were significant main effects of trial ( $F_{1,7} = 22.85$ ;  $P = 0.009$ ) and interaction between trial and time ( $F_{3,21} = 13.63$ ;  $P < 0.001$ ) for plasma concentrations of cortisol, with higher levels at Post-EX, P-EX-1h, and in EX trial than in REST trial (Figure 3).

#### Salivary Responses

For the salivary responses of saliva flow rate, sIgA concentration, sIgA secretion rate, sIgA1 concentration, and percentage of sIgA1/sIgA, there were no significant main effects of trial or time (Figure 4, 5, 6, 7, & 8).

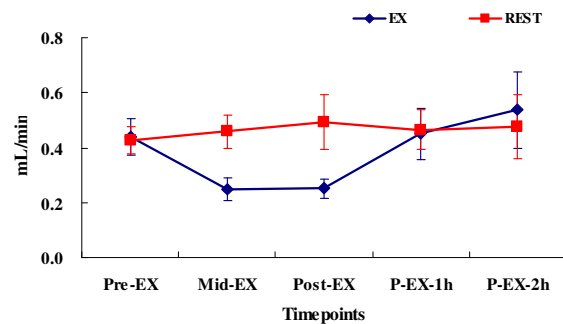


Figure 4. Changes in Saliva flow rate.

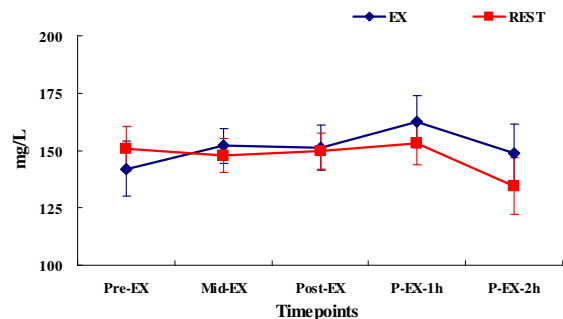


Figure 5. Changes in sIgA concentration

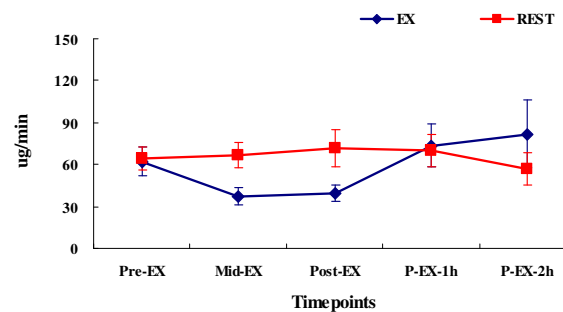


Figure 6. Changes in sIgA secretion rate.

### Discussion

The main findings of this study were that a 2-h cycling at 55% peak power significantly decreased blood glucose

concentration and increased plasma concentrations of cortisol and adrenaline. However, there were no significant effects on saliva flow rate, sIgA level, and sIgA1/sIgA ratio.

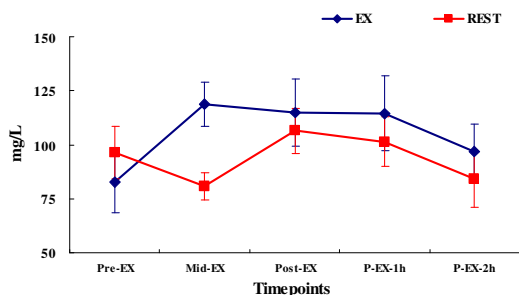


Figure 7. Changes in sIgA1 concentration.

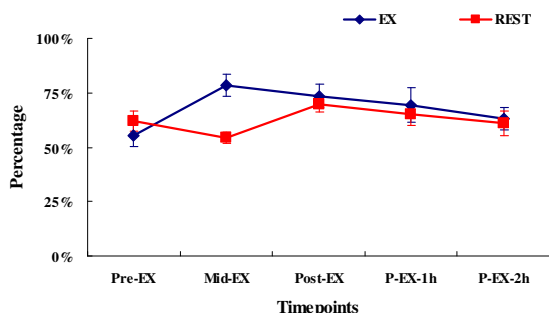


Figure 8. Changes in percentage of sIgA1/sIgA.

According to Li and Gleeson [38], the intensity of cycling at 55% peak power is equivalent to 60%  $\dot{V}O_{2\max}$  and a 2-h cycling at such intensity may oxidize around 200 g (>80% of the total available glycogen stores after an overnight fast) of carbohydrate. The reduction of glucose availability was also one of the important factors activating hypothalamic-pituitary-adrenal (HPA) activity [39] and glucoregulatory hormone secretion [40]. Therefore, it was not surprised to find a reduction in plasma glucose concentration and an increase in plasma concentrations of adrenaline and cortisol after 2 h cycling at 55% peak power in this study.

Salivary glands are innervated by both parasympathetic cholinergic nerves and sympathetic adrenergic nerves. During exercise, the sympathetic stimulation is increased and induces vasoconstriction, which limits saliva secretion rate [36]. Salivary IgA secretion has been shown to be stimulated by  $\alpha$ -adrenoceptors [41]. In the present study, the plasma adrenaline concentration was increased during exercise. However, a significant alteration in salivary responses was not found. The possible explanation may be from a concept "threshold" since Proctor *et al.* [41] suggested that the increase of sIgA secretion via elevated transcytosis from the glandular IgA pool under acute sympathetic stimulation might be induced in a dose-independent manner above a certain threshold.

Limited evidence has been revealed regarding the effect of exercise on sIgA subclasses. The results of this study indicated that an acute prolonged cycling did not affect the percentage of sIgA1/sIgA in saliva although Gregory *et al.* [34] reported that the concentrations of IgA and IgA1 in breast milk were decreased after exhaustive exercise.

In conclusion, these findings suggested that a single bout of cycling at 55% peak power for 2 h does not appear to acutely

affect oral immunity.

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